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16. ABSTRACT

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JOURNAL ARTICLE

Simplified Micro Perchlorination Method for Polychlorinated Biphenyls in Biological Samples

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Simplified methodology is presented for the micro determination of polychlorinated biphenyls (PCBs) in biological samples, by conversion to the decachlorobiphenyl (DCB) derivative. Beef adipose tissue and human milk extracts were fortified with PCB standards at 0.1–5.0 ppm, and perchlorinated with antimony pentachloride ($SbCl_5$). Several Aroclors representing various degrees of chlorine content were investigated to assess the efficiency of conversion to DCB. Samples were cleaned up on a Florisil mini column and the PCBs were quantitated by electron capture GLC. Several chlorinated pesticides which were subjected to the perchlorination procedure did not interfere. As little as 0.1 ppm PCBs in 500 mg tissue extract can be recovered at 79–99%. The background DCB content of several brands of $SbCl_5$ was determined. The levels of PCBs in human milk obtained by the perchlorination technique are compared with data acquired by electron capture gas-liquid chromatography in which the individual chlorobiphenyls in the sample are measured.

Polychlorinated biphenyls (PCBs) are among the most widely distributed anthropogenic pollutants in the environment. Detectable amounts of these compounds have been found in the majority of human tissue samples analyzed in the United States (1). PCB contamination of human milk has been reported in the United States and several foreign countries (2). These compounds have also been found in the marine life, wildlife, river water, soil, and air of the United States (1).

Determination of Aroclor® 1254 in blood has been reported (3) with quantitation based on measurement of individual peaks in the chromatogram. Metabolism of Aroclors 1242 and 1016 in rats has been studied (4) as well as residues and distribution of Aroclor 1254 in poultry and eggs (5).

Most of the analytical methodology reported has relied on measurement of individual components of PCBs and comparison with standards which closely match the chromatographic pattern of the sample. Combined gas-liquid chromatography-mass spectrometry (GLC-MS) has also been used, especially when identification of components or metabolites is desired (6).

An alternative approach to quantitation of PCBs is conversion of the multicomponent mixture to an easily measured compound—decachlorobiphenyl (DCB). Perchlorination of PCB standards to DCB with antimony pentachloride ($SbCl_5$) has been reported at the 1 μg level (7, 8) and in fish and chicken fat at about 4 ppm (7). The choice of $SbCl_5$ can be critical because of the background level of DCB in the reagent (9). Bromine present in certain lots of $SbCl_5$ can also result in the formation of bromononachlorobiphenyl (10).

This report describes the investigation of a modified perchlorination reaction and its application to the determination of PCBs in adipose tissue and human milk at levels as low as 0.04 ppm.

METHOD

Reagents and Materials

(a) *Antimony pentachloride ($SbCl_5$).*—J. T. Baker Chemical Co., Phillipsburg, NJ; Cerac/Pure, Menomonee Falls, WI; Alfa Products, Beverly, MA; Research Organic-Inorganic Chemicals (Roc-Ric), Belleville, NJ (99.99 and 99%); Matheson, Coleman and Bell (MCB), Norwood, OH.

(b) *Solvents.* — Pesticide grade hexane and $CHCl_3$.

(c) *Sodium sulfate.*—Anhydrous granular, hexane-extracted.

(d) *Sodium bicarbonate.*—10% aqueous solution, partitioned with hexane to remove contaminants.

(e) *Hydrochloric acid.*—6*N*, partitioned with hexane to remove contaminants.

(f) *Water.*—Distilled and hexane-extracted.

(g) *Florisil.*—60-100 mesh packed in mini columns, prewashed, and activated at 130°C in columns for ≥16 hr.

Use of trade names is for identification only and does not constitute endorsement by the U.S. Environmental Protection Agency.

(h) *Decachlorobiphenyl.*—99% analytical reference standard, Pesticide Repository, Environmental Protection Agency, Research Triangle Park, NC.

(i) *Polychlorinated biphenyls.*—Aroclor 1221, 1232, 1242, 1254, 1260, and 1268, Pesticide Repository.

Apparatus

(a) *Gas-liquid chromatograph.*—Tracor MT-222 equipped with ^{63}Ni electron capture detector operated in pulsed linearized mode, methane-argon (5+95) carrier gas 60 ml/min; temperatures (°C)—column 200-210, detector 250, inlet 235, and transfer line 220.

(b) *Columns.*—Borosilicate glass, 0.9 and 1.8 m × 4 mm id packed with 80–100 mesh Gas-Chrom Q coated with 5% OV-210 or 4% SE-30/6% OV-210.

(c) *Gas-liquid chromatograph/mass spectrometer.*—Finnigan Model 3200 equipped with chemical ionization source, Model 6100 data system, and 1.5 m × 2 mm id column containing 1.5% OV-17/1.95% OV-219; temperatures (°C)—column 210, source 60, transfer line 225, separator 220; electron energy 80 ev, emission current 1 ma, reagent gas methane.

(d) *Vial.*—Mini-Aktor®, 1.5 ml capacity (Applied Science Laboratories, Inc., State College, PA).

(e) *Evaporator.*—N-Evap® (Organomation Associates, Worcester, MA).

(f) *Glassware.*—Chromaflex columns size 22-7 (Kontes, Inc., Vineland, NJ).

Preparation of Florisil Column

Place small plug of hexane-extracted glass wool in tip of Chromaflex column. Add 1.6 g Florisil in small increments with gentle tapping of column stem. Add 1.6 g anhydrous, granular Na_2SO_4 to top of Florisil with tapping. Elute column with 50 ml hexane and thoroughly dry by forcing air through the column before placing in 130°C oven until used.

Perchlorination

Quantitatively transfer aliquot of cleaned up tissue extract from ≤500 mg original sample, obtained from modification of Mills *et al.* procedure (11), to Mini-Aktor vial, using 0.1 ml $CHCl_3$. Concentrate volume to ca 0.02–0.03 ml with gentle stream of nitrogen. Repeat addition of 0.1 ml $CHCl_3$ and concentration of volume twice more to remove traces of hydrocarbon solvents. (Hydrocarbon solvents react with $SbCl_5$ to form carbonaceous mass (7).) Avoid complete dryness of sample to prevent loss of lower chlorinated PCBs. Add 0.2 ml $SbCl_5$ to vial and quickly seal with Teflon cap. Place vial in sand bath maintained at 160–165°C for 16 hr.

Cleanup and Analysis

Remove vial from sand bath, let cool, and carefully remove cap. Add ca 0.5 ml 6*N* HCl to sample to deactivate excess reagent. Transfer contents to clean centrifuge tube. Rinse vial with another portion of acid and transfer rinse to centrifuge tube. Rinse vial with ca 0.5 ml hexane and transfer hexane to centrifuge tube. Repeat hexane rinse twice more. Also rinse vial cap with several drops of acid and hexane to complete transfer of sample.

Mix contents of sample tube 30 sec on vortex mixer. Let solvent phases separate and transfer hexane layer to clean tube. Add 1.0 ml hexane to sample tube and repeat partitioning and transfer of hexane. Repeat hexane partitioning once more. Add 1.0 ml water to combined hexane extracts and mix 30 sec. Discard aqueous phase and wash hexane with 1.0 ml 10% $NaHCO_3$. Transfer hexane to clean tube.

Evaporate hexane to ca 0.25 ml, using gentle stream of nitrogen. Quantitatively transfer sample to Florisil minicolumn that has been wetted with ca 10 ml hexane. Elute sample with 7 ml hexane. Adjust sample to suitable volume prior to injection into a gas-liquid chromatograph equipped with 5% OV-210 column operated at 205°C.

Determine amount of DCB in sample. Convert to desired Aroclor by multiplying DCB found by quotient obtained from average molecular weight of Aroclor divided by molecular weight of DCB (199).

Results and Discussion

With low levels of PCBs in adipose tissue, the $SbCl_5$ reagent used to perchlorinate must be carefully selected in order to minimize the contribution of background DCB by the reagent. At low concentrations, glassware must be carefully cleaned to avoid PCB contamination. After experiencing problems with excessively high recoveries at 0.1 ppm PCBs, it was determined by combined GLC-MS that a certain amount of DCB was present in blank $SbCl_5$ after perchlorination. A check of several other brands of $SbCl_5$ also revealed residual amounts of DCB. One other investigator has reported DCB in various brands of $SbCl_5$ (9). Table 1 presents our results for DCB in several commercial preparations of the reagent. One brand contained a major interfering peak that prevented quantitation of the DCB. The presence of a peak well separated from DCB, but eluting immediately after DCB, was observed in perchlorinated standards of low

Table 1. Decachlorobiphenyl in antimony pentachloride reagents

Supplier	DCB, ng/0.2 ml[a]	As Aroclor 1254, ng/0.2 ml
Baker	3	2
Cerac	2	1
Ventron	27	18
Roc-Ric (99%)	—[b]	—[b]
Roc-Ric (99.99%)	3	2
MCB	5	3

[a] Average of 4 determinations. Results were rounded to 1 digit.
[b] Could not quantitate because of interfering peaks.

Table 2. Perchlorination of adipose tissue extracts (500 mg) fortified with selected Aroclor standards

Aroclor	Fortification level, ppm	Rec., %[a]	Rel. std dev., %
1016	0.1	79.0	13.5
1016	0.2	82.6	8.71
1016	1.0	80.6	3.59
1016	5.0	76.7	1.19
1242	0.1	83.8	9.19
1242	0.2	88.6	5.87
1254	0.1	96.7	17.2
1254	0.2	98.9	7.78
1260	1.0	96.8	3.30
1260	5.0	95.5	1.47

[a] Average of 4 determinations at each indicated concentration level; mean recovery = 87.9%; 95% confidence limits for the mean = 87.9% ±19.0.

chlorine substitution such as Aroclors 1221 and 1232. This component may be bromononachlorobiphenyl (BNCB) formed from bromine in the $SbCl_5$ reagents as reported by Huckins *et al.* (10). All brands of $SbCl_5$ produced the peak. This compound does not interfere with the accurate measurement of DCB on the 5% OV-210 column.

Recovery studies were conducted by fortifying beef adipose tissue and goat milk extracts obtained with a modification of the Mills *et al.* procedure (11) with various amounts of selected PCB standards. These samples were subjected to perchlorination, cleaned up, and analyzed by electron capture GLC. Table 2 indicates the recoveries obtained. There were no compounds resulting from the lipid material which interfered with the quantitation of DCB. Cleanup by elution through a Florisil mini column removed many potentially interfering compounds. Several chlorinated pesticides (*p,p'* DDT, *p,p'*-DDE, *p,p'*-DDD, dieldrin, and heptachlor epoxide) were also subjected to perchlorination. No interferences from any of these were observed in the gas-liquid chromatograms. As shown in Table 2, the method will tolerate at least 500 mg tissue extract containing as little as 0.1 ppm

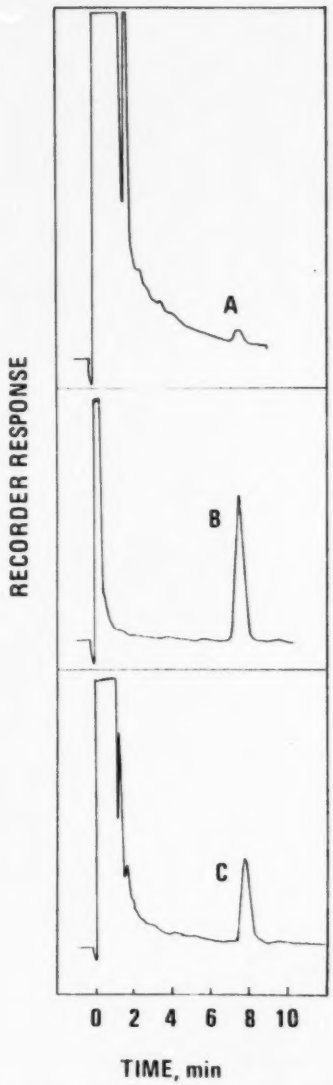

FIG. 1—Chromatograms of A, DCB extract from $SbCl_5$ reagent, 5 μl/1.0 ml injected; B, 40 pg DCB standard; C, perchlorinated fat extract (0.16 ppm as Aroclor 1254), 5 μl/6.0 ml injected; oven temperature 205°C; carrier gas flow 60 ml/min.

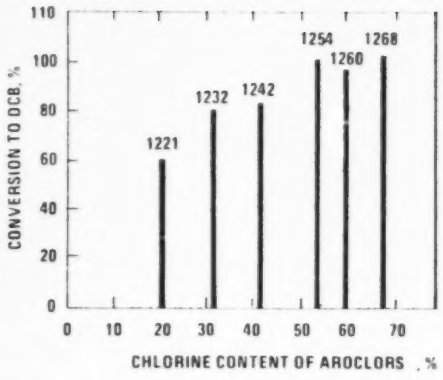

FIG. 2—Conversion of Aroclor standards (100 ng) to DCB at 160–165°C for 16 hr.

Table 3. Polychlorinated biphenyls in human milk extracts (500 mg) determined by a micro residue procedure and perchlorination

Sample	PCBs found, ppm (as Aroclor 1254)[a]	
	Perchlor.	Micro
1[b]	0.12	0.15
2[c]	0.14	0.17
3	0.05	0.04
4	0.06	0.07
5	0.08	0.09
6	0.06	0.06
7	0.06	0.06
8	0.04	0.05
9	0.14	0.19
10	0.07	0.10

[a] Reported on a whole milk basis.
[b] Goat milk fortified with 0.15 ppm Aroclor 1254.
[c] Goat milk fortified with 0.20 ppm Aroclor 1254.

PCBs to yield recoveries in the 79-99% range. Figure 1 illustrates the amount of DCB obtained from a particular brand (Cerac) of $SbCl_5$, DCB standard, and a perchlorinated sample of beef fat (500 mg) which was calculated to contain (from the DCB produced) 0.16 ppm PCBs (as Aroclor 1254).

The efficiency of conversion of various Aroclors to DCB is shown in Fig. 2. Aroclors with higher chlorine substitution were more readily converted to DCB than the lower substituted chlorobiphenyls. The difference is not great except for Aroclor 1221 (primarily monochlorobiphenyls and dichlorobiphenyls) which gives approximately 60% of the theoretical DCB yield. The decrease in recovery of DCB from lower chlorinated Aroclors may be due to the amount of bromononachlorobiphenyl formed, especially with Aroclor 1221 where several per cent of BNCB was observed to result after perchlorination. Other investigators have reported similar findings (9, 10, and V. W. Burse (1973) unpublished data, CDC, Atlanta, GA). Polychlorinated biphenyl isomers having higher percentages of

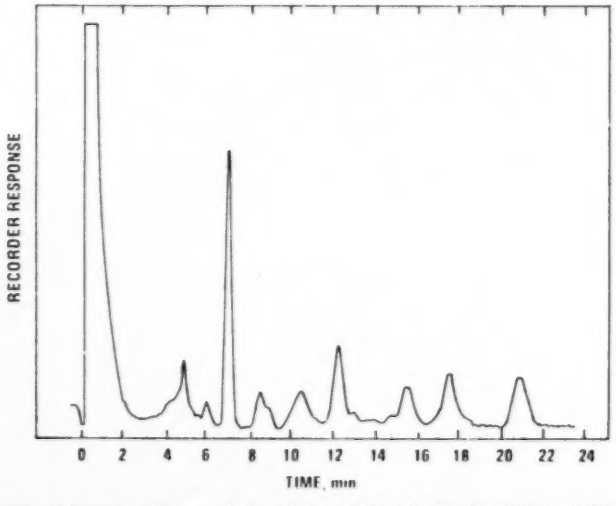

FIG. 3—Chromatogram of human milk extract: 5 μl/1.0 ml injected; 4% SE-30/6% OV-210 oven temperature 205°C; carrier gas flow 60 ml/min.

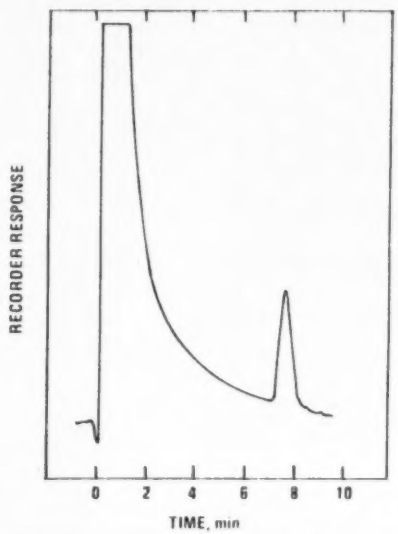

FIG. 4—Chromatogram of perchlorinated human milk extract (0.14 ppm as Aroclor 1254); 5 μl/8.0 ml injected; OV-210 column; oven temperature 205°C; carrier gas flow 60 ml/min.

chlorine substitution, such as hexachlorobiphenyls, are members of the PCB series of compounds which are likely to be stored in adipose tissue. This is because of their resistance to metabolism in the mammalian system (12).

Since all of the Aroclors have an average molecular weight less than 499 (the molecular weight of DCB), levels of PCBs expressed as a particular Aroclor will always be less than that calculated for DCB.

Human milk samples containing low concentrations of PCBs (confirmed by GLC-MS) were extracted and cleaned up with a micro multiresidue procedure (13). A silicic acid column separation, similar to that described by Cromartie *et al.* (14), was miniaturized. After quantitation by electron capture GLC, the samples were perchlorinated with $SbCl_5$. The results in Table 3 indicate reasonably good agreement at the low concentrations present. Chromatograms illustrating a human milk sample before and after perchlorination appear in Figs 3 and 4, respectively. The micro method used for the milk sample extraction and cleanup is particularly well suited for confirmatory purposes when the amount of sample is limited. The cleanup achieved allows perchlorination of the sample without excessive lipid interference with the reaction.

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